reversal tests are presented elsewhere. The resistant *C. reinhardtii* cells could present an opportunity to select the resistance gene by transformation experiments. If the resistance is due to target site alteration, which seems to be the case, this could lead to finding the target gene.

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Guttation – the basis of an assay for evaluating formulation behaviour *in vivo*

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Abstract: Guttation droplets collected from the tips of winter leaves, previously treated with a ^{14}C version of an experimental xylem-mobile fungicide (ExpF) that was known to elute readily in guttation fluid, were analysed for the presence of radiolabel. The effects of adjuvants on the elution rate was investigated and related to the known biological profile of the fungicide when used in combination with adjuvants. This method, using ExpF as a model molecule, is undergoing further development as a means of investigating formulation behaviour *in vivo*. Not all xylem-mobile fungicides elute significantly and data are presented to illustrate this.

Keywords: guttation; mobility; fungicide; adjuvant; formulation

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1 INTRODUCTION

When the dew point of air is reached, leaves can no longer lose water as vapour. To maintain transpiration under these conditions, water is lost in the liquid phase by a process called guttation. Water exudes through specialised cells called hydathodes and droplets appearing at the leaf tips or margins can contain various salts, sugars and other organic substances.[1]

In previous experiments (Harris R I, unpublished), it was noticed that an experimental xylem-mobile fungicide (ExpF) eluted in significant quantities in the guttation fluid of winter wheat seedlings. The possibility of using this molecule as a means of evaluating formulation behaviour on leaf surfaces was recognised. By incorporating ExpF in different formulation systems, and subsequently collecting and analysing guttation fluid from treated plants, it should be possible to investigate a variety of formulation properties.

This summary presents the basic methods and preliminary data using a model acetone-based formulation system. Of particular significance for further evaluation are release profiles of encapsulated formulations.

2 MATERIALS AND METHODS

2.1 Active ingredients

The fungicides used were [^{14}C] ExpF, Specific activity 6.5 MBq mg^{-1}, and [^{14}C]fluquinconazole, Specific activity 6.2 MBq mg^{-1}. Final application solutions contained the fungicide at 0.5 g litre^{-1}, composed entirely of the radiolabelled molecule. The activity of the final application solutions was approximately 3.8×10^3 Bq µl^{-1} for [^{14}C]ExpF and 3.2×10^3 Bq µl^{-1} for [^{14}C]fluquinconazole.

2.2 Adjuvants

Two adjuvants, Adj1 and Adj2, of known widely differing uptake activation properties were used. Adj1 enhances the uptake of lipophilic, high-melting-point molecules into cereal foliage, whilst Adj2 is better suited to less lipophilic molecules of lower melting points.

2.3 Plants

Winter wheat (*Triticum aestivum* L cv Avalon) seedlings, grown in John Innes compost and maintained at a nominal 20 °C and 16 h photoperiod, were used at the one to two fully expanded leaf stage.

2.4 Application and sampling

The fungicides (0.5 g litre^{-1}) in acetone + water (85 + 15 by volume), together with the appropriate

Figure 1. Expermental layout.

adjuvant, were used in the following combinations: 1. ExpF + Adj1 2. ExpF + Adj2 3. Fluquinconazole + Adj1.

Ten 0.2-µl droplets of each application solution were applied to the mid portion of the upper surface of the first leaf of winter wheat seedlings at the one- to two-leaf stage. There were three seedlings per pot and five replicate pots per treatment. Immediately after application, the tip of each treated leaf was passed through a slit in a Parafilm cap fitted over the end of a pre-weighed glass scintillation vial. All three treated leaves per pot were led into a single vial placed equidistant from each plant (Fig 1). Guttation commenced at the end of each light period and vials were changed and weighed periodically soon after a lighting period commenced and guttation ceased. After allowing the water to evaporate, scintillation cocktail (naphthalene + 2,5-diphenyloxazole in dioxane, $100 + 5\,g\ litre^{-1}$; 10 ml) was dispensed to each vial and radiolabel quantified by counting on an Intertechnique SL30 Liquid Scintillation Spectrometer.

3 RESULTS AND DISCUSSION

Each treatment had a distinctive recovery profile in guttation fluid (Fig 2). When ExpF was mixed with the uptake-promoting adjuvant Adj1, large quantities of radiolabel were quickly eluted in the guttation fluid, 15% of the applied label being recovered over 24 h, three days after application. Conversely, when ExpF was mixed with Adj2, an adjuvant that did not promote uptake, recoveries of radiolabel in guttation fluid were considerably lower, reaching a maximum single loss of 1.5% over a 24 h period four days after application. By seven days after application, losses

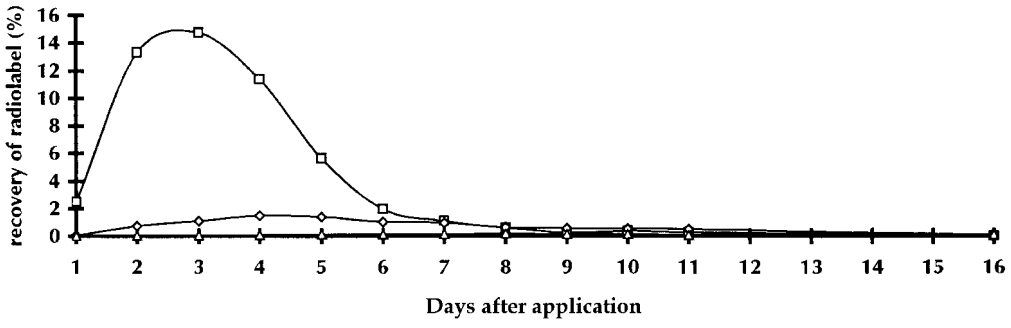

Figure 2. Recovery of radiolabel in guttation fluid after application of [^{14}C] ExpF and [^{14}C] fluquinconazole to the foliage of winter wheat. (□) ExpF + Adj1, (◊) ExpF + Adj2, (△) Fluquinconazole + Adj1.

from both ExpF-based treatments were almost identical.

Not all xylem-mobile molecules behave in the same way and this is illustrated by a comparison between ExpF and fluquinconazole when both were mixed with Adj1. Fluquinconazole was recovered only in very small quantities, the single greatest recovery being 0.17% over a 24h period. These recoveries are considerably less than those already described for ExpF when mixed with the same adjuvant. That recovery in guttation fluid is not solely related to the level of uptake into the plant is supported by data from other experiments (Harris RI unpublished) where the uptakes of both fluquinconazole and ExpF were very similar when mixed with Adj1 (60 and 69%, respectively, 24h after application).

Using ExpF as a model compound it should be possible to investigate the behaviour of other formulation systems on target crops. A specific example of the use of this system is the evaluation of encapsulated formulations. It is possible to prepare capsules with a range of properties, one of these being ease of capsule breakdown, resulting in the release of the encapsulated compound. This can be monitored using artificial surfaces, but there is now the possibility of doing studies *in vivo* using the model described above.

Information about the loss of fungicide, insecticide and herbicide molecules in guttation fluid can also help explain biological performance against target species. For example, the activity of ExpF against *Erysiphe graminis* DC f sp *tritici* Marchal, (the causal organism of powdery mildew of wheat) was enhanced when mixed with Adj2 but not Adj1 (Moss, N A pers. comm.). From the results discussed above it is possible that a biologically significant proportion of the applied dose of ExpF, when mixed with Adj1, was lost through guttation, resulting in an inferior performance compared with the mixture with Adj2. Life-cycle studies showed ExpF to be more effective as a protectant than curative fungicide and that the early stages of pathogen development were most affected. A formulation optimising surface retention rather than foliar uptake would, therefore, be more appropriate.

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Synthesis and insecticidal activity of 4-perhaloalkoxy (or thioalkyl) benzophenonehydrazone derivatives

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Abstract: Benzophenonehydrazone derivatives containing a mesylate or triflate substituent are known to exhibit insecticidal activity. In the present study, such substituents have been replaced by perhaloalkoxy groups. High levels of activity against lepidopteran pests were observed in greenhouse trials. For optimum activity, the substituents should be relatively small. In semi-field trials, however, none of the compounds tested showed sufficient persistence to warrant further development.

Keywords: benzophenonehydrazones; perhaloalkoxy substitution; insecticidal activity; lepidoptera; coleoptera; structure–activity relationships

1 INTRODUCTION

Amidinohydrazones have long been known for their pharmacological properties (eg for use against malaria).[1] Compounds of structure type **1** (Fig 1) were identified in the 1970s, as a class of insecticide active against lepidopteran and coleopteran pests.[2,3] Interest in this chemistry re-intensified in the 1990s. During our own investigations, we found that the triflate or mesylate substituent in **1** (Fig 1) could be replaced by a perhaloalkoxy substituent, whilst still preserving the biological activity. An optimisation programme was started to prepare compounds of structure type **2** (Fig 1) with the goal of improving the insecticidal properties and identifying compounds which could provide cost-effective control of lepidopteran and coleopteran pests in cotton and vegetables.[4]

The present communication gives an outline of the synthetical methodology used, together with selected biological data and structure–activity relationships.

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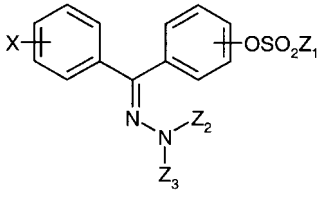

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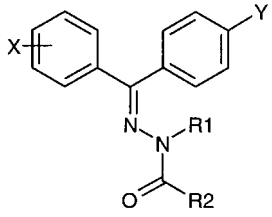

2

X = 4-Cl, Br, F; R1 and R2 = H, alkyl, aryl
Y = OCF_3, OCF_2Br, OCF_2Cl, OCF_2CF_2Br, SCF_3

Figure 1. General structures of the compounds discussed in the text.